Curr. Res. Health Sci., 2(2): 1–5, 2024. DOI: 10.58613/crhs221

Current Research in Health Sciences

Homepage: https://www.gpub.org/journal-details.php?journal-id=47

ISSN (E): 2584-1084

Review Article

Identifying New Strain From Family Tenebrionidae, Order: Coleoptera Using COI Gene Sequencing

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Article Info

Keywords: *Biodiversity, COI Sequencing, Tenebrionidae*

Received: *29 June 2024*
Accepted: *24 August 2024*
Published: *7 September 2024*

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Abstract

Renowned for its ecological versatility and adaptability, the family Tenebrionidae includes more than twenty thousand species. These darkling beetles occur in almost every type of habitat, from the humid forests to arid deserts, that represents evolutionary success. Key morphological characteristics include a hard body and elytra, to protect against extremes in the environment and against predators. Coupled with this, the nocturnality and burrowing behavior coupled with being detritivores underpin their importance in nutrient cycling and generally in ecosystem health. Notable examples of species include Tribolium castaneum, Eleodes spp. and Tenebrio molitor, where their specific environmental resistances have been known for a long time. Advances in molecular phylogenetics, especially the sequencing of COI, have recast taxonomy and improved knowledge on the Tenebrionidae by uncovering cryptic diversity and resolving phylogenetic relationships. Studies on DNA barcoding and environmental DNA have improved the state of species identification and monitoring, allowing new insights into biogeographical patterns of these beetles and their conservation needs.

1. Introduction

Family Tenebrionidae is popularly known as the darkling beetles and represents one of the most diverse families within the order Coleoptera. It consists of over twenty thousand species of wide distribution in the world, showing great adaptability to different ambits, from the densest forests to the most arid deserts [1]. Members of this family are mostly characterized by having a hard body, and many species have a hard exoskeleton that helps them in survival under adverse conditions. Their elytra are mostly fused, thus protecting them further from drying out or becoming prey. Morphologically, the beetles of the family Tenebrionidae show a great diversity in terms of body shape and size and color, although many have a dark or metallic sheen as a characteristic feature [2]. A part from their physical robustness, beetles of this family also have many behavioral and physiological adaptations that have evolved and play a role in adapting in extreme environments.

Ongoing climatic change such as the recurrence and intensity of extreme events, rising of temperature and, may effect on species already living near the upper limits of their climate tolerance. One of the characteristics of the dark beetles is their inability to fly in the sand dunes. Although this characteristic may be important in this type of environment, it reduces their ability to spread in this type of desert environment. Based on the above, the possibility of tracking them and studying their characteristics in these conditions is easy. In fact, due to this adaptation, their presence is limited to specific and relatively small areas on the sandy coasts. As a result, the numbers of these insects increase in these specific areas, especially when suitable conditions are available, such as high temperatures in addition to the inability to move to other places [3].

For example, most species are nocturnal burrowers to avoid the severe circumstances seen in all desert settings during the day. The family's ability is only matched by its members' dietary habits; all Tenebrionidae beetles are detritivores, meaning they feed on decaying organic

matter, and hence play a crucial role in nutrient cycling throughout any environment [4].
In reaction to higher temperatures, darkling beetles create heat shock proteins, heat resistance enzymes, and other cryoprotectant compounds [5]. Some possess reflecting elytra. certain have relatively lengthy legs for their body size, however certain species exhibit ”stilting” behavior at high temperatures, in which their body is kept separate from touching with warm Sand and cools by greater convective cooling [6]. This ecological role is particularly important in nutrient-poor environments like deserts, where these beetles help sustain the ecosystem's health by breaking down plant and animal matter.
Tenebrionidae spp. that live in the extreme portions of central Asia with an extremely continental environment, and temperatures measured in soil in these natural locations looked to be lower than or near the beetles' cold resistance threshold. As a result, darkling beetles could only overwinter in regions with thick snow, such as hollows, shrubs, and huge crops [7]. This suggests that cold-resistant darkling beetles could not have existed in the late Pleistocene due to the significantly colder environment and were not found in ancient fauna [8].

2. General Characteristics

Morphology

Darkling beetles vary significantly in appearance, but they are typically described as having a strong body that is mainly flattened. These beetles have stiff elytra that protect the hind wings and are often drab in color, ranging from brown to black; however, certain species, such as, have a silver look [2, 9]. One distinguishing trait of the Tenebrionidae is the lack of a clear distinction between the head and thorax, despite the presence of protective glands that emit chemicals to repel predators [10].

Ecology and Behavior

Tenebrionidae are largely detritivorous, feeding on decaying plant debris, fungus, and animal matter, and play a crucial role in nutrient cycling within an ecosystem [4]. The Adesmia metallica and Eleodes obscura species are most suited to live in desert settings, thanks to their nocturnal habits and burrowing ability [11]. These organisms play an important role in nitrogen cycle in their environments [12, 13].

Tenebrionidae: Key Species and Environmental Adaptation

Tribolium castaneum (Red Flour Beetle)

One of the most well-known species within the Tenebrionidae family is Tribolium castaneum, commonly known as the red flour beetle, it is Indo-Australian and lives in temperate zones. It also called red flour beetle, and is a worldwide pest of grains that are stored and the most common insect problem in grain handling and storing operations [14]. This pest's short lifespan allows it to compete for limited resources, live in bad environments, and adapt to changing environmental factors [15]. This species' adults exhibit strong dispersion behavior, especially at large numbers of individuals. They also have excellent flight and behavioral abilities [16].

Tenebrio molitor

Tenebrio molitor also called yellow mealworm is economically important member in the family of Tenebrionidae, is extensively used as a source of food for mammals, in research, and as a developing source of sustained protein in the diet of people [17]. They function as decomposers, consuming decaying plant waste and deceased insects specially, in their natural habitat [18]. It can swiftly convert poor compenant waste from plants into meals high in calories, protein, and lipids [19]. This insect may flourish in a variety of climates, from warm to subtropical, also it may thrive for a long duration of time lacking sustenance and is extremely resilient to drying. The main reason that gives this insect the advantage of resisting drought and growing normally in these difficult conditions is the ability to extract water from several organic compounds [20].
T. molitor's life cycle comprises egg, larva, pupa, and adult stages; the female can deposit 250-1000 eggs, with a median of 400-500 [21].

Eleodes spp. (Stink Beetles)

The genus Eleodes, commonly referred to as stink beetles, includes several species highly adapted to desert environments, particularly in North America. These beetles are named for their defensive behavior of releasing a foul-smelling secretion when threatened. Environmental Resistance: Eleodes species are specially adapted to survive in extreme desert conditions, where temperatures can fluctuate dramatically between day and night. They have evolved behaviors such as burrowing into the sand to avoid extreme heat and reducing activity during the hottest parts of the day. Their dark coloration is thought to play a role in thermoregulation, helping them absorb and retain heat in cooler desert nights [6, 22].

DNA Barcoding for Species Classification

COI barcoding (cytochrome C oxidase I) was utilized to analyze the abundance of species belong to family of Tenebrionidae in Mediterranean settings, that are amongst the most varied and unique of any place [23]. The results revealed multiple species, including new records for the region, highlighting the importance of using molecular diagnostics (COI gene) to monitor and conserve bio diversity.

3. Previous Studies on The Use Of Molecular Methods And Genetics In The Study of Tenebrionidae Species

DNA barcoding technique was created to aid in the identification of cryptic and undescribed species. The mitochondrial cytochrome c oxidase I region (mt DNA COI) was used for insect taxa barcoding analysis. COI's ability to accurately identify species, uncover cryptic variation [24]. Also analyze phylogenetic relationships has broad implications for taxonomy, biology maintenance, and environmental studies.

For the initial time, researchers were able to construct a molecular evolutionary tree for the tribe Sepidiini using DNA sequence data from the following five loci: COI, COII, and additional genetic loci. This study produced an evolutionary analysis of thirty-nine species representing Sepidiini and a supplementary morphological assessment [25, 26]. demonstrated that T. castaneum and T. confusum are genetically separate regardless of their extremely comparable size and shape, and theareas of COI and Cytb are extremely helpful to assess genetic variation and evolutionary relationships between species, particularly morphologically comparable species. Furthermore, the molecular phylogeny does not fully align with the evolutionary connections derived from morphological data [26]. Guerrero and colleagues created a novel classification of Stenochiinae in Chile, where this insect exhibits extraordinary morphological traits. A new genus and two species of Stenochiinae are reported using phylogenetic analyses of the COI in addition of (28S, 18S, 16S) sequences, as well as analysis of general morphological. Stenochiinae, a subfamily of Tenebrionidae with a primarily tropical distribution, has received little attention, and taxonomy efforts to appreciate its full variety have been limited [27, 28]. determined the mitochondrial genomes of various Tenebrionidae species, including Anatolica potanini, Melanesthes exilidentata, and Myladina unguiculina, and conducted a comparative study of the mitochondrial genomes to define the family's evolutionary traits. The tenebrionid mitochondrial genomes were substantially maintained in terms of genome size, gene order, base composition, and codon use [28].

The genus Gnaptor Brulee exists in Southeast European and countries of Asia.In terms of clasification, four species have been identified: Gnaptor Boryi, G. Prolixus, G. Spinimanus, and G. Medvedevi depending on morphological characteristics. Gkontas and others employed two separate mitochondrial genetic markers cytochrome c oxidase subunit 1 and 16S to study the connections between the populations of the species G. boryi in Greece, contrast them to the existing classification, and speculate about its biogeographic origin, They highlighted the existence of three backed up lineages, two of which relate to G. boryi and one to G. prolixus [29]. Gnaptorina Reitter is the second largest and most abundant genus in the darkling beetle subtribe Gnaptorinina (Family: Tenebrioninae), with thirty-eight species or subspecies based on their physical traits.

Some scholars suggested a new classification for Gnaptorina.The phylogenetic tree of the genus was reconstructed on the basis of three mitochondrial genes, as well as one nuclear gene. This phylogenetic tree was utilized to study the origins and variety of Gnaptorina species. Several genetic species delimitation approaches were employed to reconsider the species situation in Gnaptorina and the phylogenetic divisions of the genus. Three primary groupings have been found, The monophyletic subgenus Boreoptorina is thought to be related with the more species rich subgenus Hesperoptorina, and G. dongdashanensis has been shifted from Hesperoptorina to the subgenus Gnaptorina [30].

Biogeographical studies utilizing COI data, such as those conducted by [31], provide insights into how historical climatic and geological events have shaped the distribution and diversification of Tenebrionidae species. Understanding these biogeographical patterns is essential for predicting how ongoing environmental changes may impact these beetles and their habitats.

The application of environmental DNA (eDNA) and metabarcoding techniques, as demonstrated by [32], offers a non-invasive method for monitoring Tenebrionidae populations. These innovative approaches enable researchers to assess beetle diversity and distribution in challenging ecosystems where traditional sampling methods are difficult [32].

4. Conclusion

Tenebrionidae family have evolved particular adaptations to the extreme climatic conditions including high temperatures and aridity. Tenebrionidae (darkling beetles) are one of the most taxonomically varied beetle families. Since they may be observed in a range of settings, the family is mostly renowned for their xerophilous habitat. Several severely dry habitats. These adaptations include specific anatomical characteristics such as long legs and a sub-abdominal cavity, specific life cycles, ecological specialization, and daily and seasonal activity patterns that fluctuate in response to temperature variations. Many members of the Tenebrionidae family have been successfully identified using molecular approaches, including DNA barcoding and species-specific PCR. DNA barcoding has been utilized as a highly successful approach for distinguishing species from distinct groupings of organisms The cytochrome oxidase subunit (COI) gene in mitochondrial DNA is commonly used to identify species belonging to the Tenebrionidae family. These two methods have been utilized to determine several taxa in this family and offer thorough information on their taxonomy, relatedness, and biogeography.

The combination of genetic tools and traditional ecological methods has resulted in the categorization, characterization, and preservation of the vast majority of species in this family.

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